REMARKS

Claims 1-89 are pending. Applicants have canceled Claims 1-89 in favor of new Claims 90-136. Applicants note that the amendments made herein are intended to further the their business interests and the prosecution of the present application in a manner consistent with the Patent Business Goals (P.B.G.)[1] while preserving the right to prosecute the original, or similar, claims in the future. None of the claim cancellations or additions made herein are intended to narrow the scope of the claims within the meaning of *Festo*[2] or related cases. None of the claim cancellations or addition made herein add new matter. Entry of the new claims and remarks is respectfully requested.

The Specification has been amended to correct typographical errors and to comply with the Examiner's request that the nucleic acid sequences recited in Figures 2, 11A, 11B, 15, 16A, 16B, 17B, 17C, 18A, 18B, 18C, 18D, 20A, 20B, 22, 24, 26, 29A, 29B, 31, 37A-C, 38A-C, 39, 40-42, 43A, and 43B be identified in the Sequence Listing. Applicants respectfully submit that many of the allegedly non-identified nucleic acid sequences merely provide portions of larger previously identified sequences. The MPEP allows applicants to recite portions of previously identified nucleic acid sequences (*e.g.*, residues 14 to 243 of SEQ ID NO: 23) without assigning the portions separate sequence identification numbers. (MPEP 24422.03). Changes have been made were appropriate. These amendments do not add new matter.

In conformity with current U.S. Patent and Trademark Office rules set forth in 37 C.F.R. §1.121 *et seq*. Applicants have attached hereto at Appendix 1 a sheet entitled "Version With Markings To Show Changes Made" to present the various changes made to the Specification (as set forth under 37 C.F.R. §1.121(b)(1)(i-iii)), and the pending Claims (as set forth under 37 C.F.R. §1.121(c)(1)(i-ii)). For the Examiner's convenience, Applicants have attached a clean version of the entire set of pending Claims as amended by this communication. (*See*, Appendix 2).

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[1] 65 Fed. Reg. 54603 (September 8, 2000).

[2] *Festo Corp. v. Shokestu Kinzoku Kogyo Kabushiki Co.*, 234 F.3d 558 (Fed. Cir. 2000) (en banc), *cert. granted.*

Dated: January 18, 2002

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Appendix 1

Version With Markings To Show Changes Made

In The Specification:

Please amend the paragraph beginning at page 56, line 6 to read as follows:

--Figures 2A-2D provide [a] schematic [of representation] representations of a segment of the katG gene from M. tuberculosis. Depending on the sequence, the segment of the DNA can form the stem-loop structures depicted in 2C and 2D. The arrows in 2C and 2D show the sites that are cleaved when these structures are treated by the structure specific Cleavase® I nuclease. The black bar to the left of each structure indicates the region to which the katG probe would bind, with the pointed kink in the bar indicating a site of mismatch between the probe and the katG target. [Figures 2A-2D show portions of SEQ ID NOS:1-4 (structures 2A-2D, respectively)] Figure 2A shows a polynucleotide that spans the region from residues 29 to 391 of SEQ ID NO:1. Figure 2B shows a polynucleotide that spans the region from residues 29 to 391 of SEQ ID NO:2. Figure 2C shows a polynucleotide that spans the region from residues 29 to 391 of SEQ ID NO:3. Figure 2D shows a polynucleotide that spans the region from residues 29 to 391 of SEQ ID NO:4.--.

Please amend the paragraph beginning at page 56, line 12, to read as follows:

--Figure 3 shows at left a fluorescence imager scan of the cleavage patterns generated using the CFLP® method on the katG substrates. The letters above the lanes indicate that these DNA fragments contain to the corresponding structures [diagrammed] diagramed in [Figure 2] Figures 2A-2D. An arrow indicates the 37 nucleotide (nt) product of cleavage at the site indicated by the arrows in [Figure 2] Figures 2C-2D. The graph at the right depicts the fluorescence intensity measured when each of the molecules depicted in [Figure 2] Figures 2A-2D was complexed to the katG capture probe and bound to a solid support in a structure probing assay.--.

Please amend the paragraph beginning at page 57, line 15, to read as follows:

--[Figure] Figures 11A and 11B show schematic representations of the capture oligonucleotides used in these studies. While are were tested with all three of the test molecules depicted in Figure 10, for convenience they are shown aligned with their complementary regions in test molecule #80 (SEQ ID NO:39).--. Figure 11A shows probe molecules Nos. 2, FD91, 80, 78, 4, 79, and 116-188 which correspond, respectively, to SEQ ID NOS: 51, 50, 39, 42, 43, 44, 47, 48, and 49. Figure 11B shows probe molecules Nos: 79, 114, and 115, which correspond, respectively, to SEQ ID NOS:44-46.--.

Please amend the paragraph beginning at page 58, line 5 to read as follows:

--Figure 15 shows an alignment of four 244 nt segments of HCV, representing types 1a (SEQ ID NO: 124), 1b (SEQ ID NO: 125), 2a/c (SEQ ID NO: 126) and 3a (SEQ ID NO: 127). Type 1a is shown in its entirety, while only the differences are indicated for the other types. Cleavage sites generated by CFLP® cleavage are indicated by vertical lines along the sequence, with the weakest cleavage sites shown as broken lines.--.

Please amend the paragraph beginning at page 58, line 10 to read as follows:

--Figure 16A and 16B show schematic diagrams of two possible secondary structures for a 244 nt fragment (SEQ ID NO: 128) derived from HCV type 1a.--.

Please amend the paragraph beginning at page 58, line 15 to read as follows:

--Figure 17B shows schematic diagrams of two of the predicted structures for a region from residues 70 to 244 of the 244 nt amplicon derived from HCV type 1a (SEQ ID NO: 128). The CFLP® data indicates that the target DNA assumes multiple conformations in solution, each contributing to the cleavage pattern (Brow *et al.*, *supra*).--.

Please amend the paragraph beginning at page 58, line 19 to read as follows:

--Figure 17C shows schematic diagram of three bridging oligonucleotides designed two interact with the predicted structures (residues 84 to 213, and residues 110 to 204, respectively, of SEQ ID NO: 128) for this region "b," "m," and "n" (SEQ ID NOS:53, 64, and

65). The regions that are complementary as aligned to the target are indicated by a black line between the strands.--.

Please amend the paragraph beginning at page 58, line 23 to read as follows:

--[Figure] Figures 18A-D show schematic diagrams of the predicted structures for a region of the 244 nt amplicon derived from HCV types 1a (residues 70 to 213 of SEQ ID NO: 124), 1b (residues 46 to 213 of SEQ ID NO: 125), 2a/c (residues 77 to 213 of SEQ ID NO: 126) and 3a (residues 77 to 213 of SEQ ID NO: 127), respectively. In Figures 18 B-D the bases that differ from the type 1a sequence are shown in bold. Each is aligned with bridging oligonucleotides "b," "i," "j," "k," "c," and "d" of six different designs (SEQ ID NOS:53, 54, 55, 56, 57, and 58). The regions that are complementary as aligned to the target are indicated by a black line between the strands. The 3' terminal contact sequence of each probe (excepting "c") is complementary to eight contiguous target bases upstream of the right most stem, but representation of the small central stem prevents showing this alignment.--.

Please amend the paragraph beginning at page 59, line 6 to read as follows:

--Figure 20A shows a schematic diagram of a structure in the amplicon derived from HCV type 1a (residues 136 to 213 of SEQ ID NO: 124) aligned with non-bridging probes "a" (SEQ ID NO: 52) and "e" (SEQ ID NO: 59) and bridging probes "b" - "d" (SEQ ID NOs: 53, 57 and 58, respectively). The regions that are complementary as aligned to the target are indicated by a black line between the strands.--.

Please amend the paragraph beginning at page 59, line 10 to read as follows:

--Figure 20B shows a schematic diagram of a structure in the amplicon derived from HCV type 1a (residues 136 to 213 of SEQ ID NO: 124) as it might be expected to pair with the fully complementary non-bridging oligonucleotide "a" (SEQ ID NO:52). The regions that are complementary as aligned to the target are indicated by a black line between the strands.--

Please amend the paragraph beginning at page 59, line 20 to read as follows:

--Figure 22 shows a schematic diagram of a structure in the amplicon derived from HCV type 1a (residues 136 to 213 of SEQ ID NO: 124) aligned with non-bridging probes "a" and "e" and bridging probe "b" (SEQ ID NOS:52, 53, and 59, respectively). The regions that are complementary as aligned to the target are indicated by a black line between the strands.--

Please amend the paragraph beginning at page 60, line 1 to read as follows:

--Figure 24 shows a schematic diagram of a structure in the amplicon derived from HCV type 1a (residues 136 to 213 of SEQ ID NO: 124) aligned with non-bridging probes "a" and "e" and bridging probes "b" - "d" and ligation oligonucleotide "f" (SEQ ID NOS:52, 59, 53, 57, 58, and 62, respectively). The regions that are complementary as aligned to the target are indicated by a black line between the strands.--.

Please amend the paragraph beginning at page 60, line 11 to read as follows:

--Figure 26 shows a schematic diagram of an unstructured synthetic target "S.T." (SEQ ID NO:63) aligned with non-bridging probes "a" and "e" and bridging probes "b" - "d" and ligation oligonucleotide "f" (SEQ ID NOS:52, 59, 53, 57, [and] 58, and 62, respectively). The regions that are complementary as aligned to the target are indicated by a black line between the strands.--.

Please amend the paragraph beginning at page 60, line 26 to read as follows:

--Figures 29A and 29B [shows a] show schematic [diagram] diagrams of either a structure in the amplicon derived from HCV type 1a (residues 136 to 213 of SEQ ID NO: 124), or an unstructured synthetic target "S.T." (SEQ ID NO:63) respectively, aligned with non-bridging probes "a" and "e", bridging probes "b" - "d" and invasive cleavage probe "g" [(SEQ ID NOS: 52, 53, 57, 59, and 58, respectively)] (SEQ ID NOS: 52, 58, 59, 53, 57, and 60, respectively). The regions that are complementary as aligned to the targets are indicated by a black line between the strands.--.

Please amend the paragraph beginning at page 61, line 7 to read as follows:

--Figure 31 shows a schematic diagram of a structure in the amplicon derived from HCV type 1a (residues 136 to 213 of SEQ ID NO: 124) aligned with bridging probe "b" (SEQ ID NO:53) and invasive cleavage probe "h" (SEQ ID NO:61). The regions that are complementary as aligned to the target are indicated by a black line between the strands.--.

Please amend the paragraph beginning at page 62, line 7 to read as follows:

--Figure 37A shows two schematic diagrams of two possible secondary structures for a 128 nucleotide fragment (SEQ ID NO: 72) derived from the *rpoB* gene of *M. tuberculosis*.--.

Please amend the paragraph beginning at page 62, line 10 to read as follows:

--Figure 37B shows four schematic diagrams; one is of the stem predicted to fold when nucleotide 62 of the *rpoB* amplicon is basepaired to nucleotide 114 (residues 54 to 122 of SEQ ID NO:72); three variant molecules, indicated as 1 (SEQ ID NO: 88), 2 (SEQ ID NO: 90), and 3 (SEQ ID NO: 92), are also depicted.--.

Please amend the paragraph beginning at page 62, line 13 to read as follows:

--Figure 37C shows a schematic diagram of a structured site in the amplicon (residues 54 to 122 of SEQ ID NO:72) derived from the *rpoB* gene of *M. tuberculosis* having a basepair between nucleotides 62 and 114, aligned with bridging probes having different spacer regions (SEQ ID NOS:106, 105, 107, 108, and 109, respectively). The regions of the target that are complementary to the probes are indicated by a black line below the target structure. A graph depicts the fluorescence signal measured after the solid support capture of this amplicon by the indicated probes. The numbers identifying the probes used in each capture test are indicated above each bar and the spacer in each probe is indicated below each bar. The fluorescence signal is shown on the left of the panel as a percentage of the signal measured in experiments using a linear (non-bridging) control probe for capture of this target.--.

Please amend the paragraph beginning at page 62, line 24 to read as follows:

--Figure 38A shows schematic diagrams of a three structured sites in the amplicon derived from the *rpoB* gene of *M. tuberculosis* aligned with bridging probes 17 -20 (SEQ ID NOS:110, 111, 112, and 113). In particular, the top left structure represents residues 45 to 126 of SEQ ID NO: 72 and the alignment of bridging probe SEQ ID NO: 110. The Top right structure represents residues 61 to 118 of SEQ ID NO: 72 and the alignment of bridging probe SEQ ID NO: 111. The bottom left structure represents residues 67 to 128 of SEQ ID NO: 72 and the alignment of bridging probes SEQ ID NOS: 112 (top) and SEQ ID NO: 113 (bottom), respectively. The regions that are complementary as aligned to the target are indicated by a black line between the strands. A graph depicts the fluorescence signal measured after the solid support capture of these amplicons by the indicated probes. The numbers identifying the probes used in each capture test are indicated below each bar, and the fluorescence signal is shown on the left of the panel as a percentage of the signal measured in experiments using a linear (non-bridging) control probe for capture of these targets.--.

Please amend the paragraph beginning at page 63, line 3 to read as follows:

--Figure 38B shows schematic diagrams of two structured sites, residues 70 to 114, and residues 5 to 95 of SEQ ID NO: 72, respectively, in the amplicon derived from the *rpoB* gene of *M. tuberculosis* aligned with bridging probes 78-106 and 63-87 (SEQ ID NOs:114 and 115, respectively). The regions that are complementary as aligned to the target are indicated by a black line between the strands. A graph depicts the fluorescence signal measured after the solid support capture of this amplicon by the indicated probe. The numbers identifying the probes used in each capture test are indicated below each bar, and the fluorescence signal is shown on the left of the panel as a percentage of the signal measured in experiments using a linear (non-bridging) control probe for capture of this target.--.

Please amend the paragraph beginning at page 63, line 13 to read as follows:

--Figure 38C shows schematic diagrams of three structured sites in the amplicon derived from the *rpoB* gene of *M. tuberculosis*, residues 76 to 110, residues 49 to 119, and residues 54 to 122 of SEQ ID NO: 72, respectively, aligned with bridging probes 84-102, 57-119 or 84-102 (SEQ ID NOs:116, 117, and 118, respectively). The regions that are

complementary as aligned to the target are indicated by a black line between the strands. A graph depicts the fluorescence signal measured after the solid support capture of this amplicon by the indicated probe. The numbers identifying the probes used in each capture test are indicated below each bar, and the fluorescence signal is shown on the left of the panel as a percentage of the signal measured in experiments using a linear (non-bridging) control probe for capture of this target.--.

Please amend the paragraph beginning at page 63, line 23 to read as follows:

--Figure 39 shows schematic diagrams of three possible structures "a" (residues 54 to 122 of SEQ ID NO: 72), "b" (residues 54 to 121 of SEQ ID NO: 72), and "c" (residues 55 to 95 of SEQ ID NO: 72) formed by the amplicon derived from the *rpoB* gene of *M. tuberculosis*. Each of these three structures could cause CFLP® cleavage 62 to 63 nucleotides from the 5' end of this fragment, contributing signal in this region of the CFLP® gel pattern.--

.

Please amend the paragraph beginning at page 63, line 28 to read as follows:

--Figure 40 shows a schematic diagram of structure "b" from Figure 39 (residues 54 to 121 of SEQ ID NO: 72) aligned with a bridging probe (SEQ ID NO:118) that could create a four-way junction. A graph depicts the fluorescence signal measured after the solid support capture of two different sized amplicons by this probe. The fluorescence signal is shown on the left of the panel as a percentage of the signal measured in experiments using a linear (non-bridging) control probe for capture of these targets.--.

Please amend the paragraph beginning at page 64, line 3 to read as follows:

--Figure 41 shows schematic diagrams of structure "b" from Figure 39, either unaltered (residues 54 to 121 of SEQ ID NO: 72), or truncated and mutated (residues 54 to 113 of SEQ ID NO: 92) to destabilize the shorter stem. Also depicted is bridging probe 62-98 (SEQ ID NO:119), designed to hybridize across the longer remaining stem, and a graph depicting the fluorescence signal measured after the solid support capture of the shortened amplicon by the indicated probe. The fluorescence signal is shown on the left of the panel as a percentage of

the signal measured in experiments using a linear (non-bridging) control probe for capture of this target.--.

Please amend the paragraph beginning at page 64, line 11 to read as follows:

--Figure 42 shows a schematic diagram of structure "c" from Figure 39 (residues 55 to 95 of SEQ ID NO: 72) aligned with bridging probe 63-87 (SEQ ID NO:115), and a graph depicting the fluorescence signal measured after the solid support capture of three different sizes of amplicon by the indicated probe. The fluorescence signal is shown on the left of the panel as a percentage of the signal measured in experiments using a linear (non-bridging) control probe for capture of these targets.--.

Please amend the paragraph beginning at page 64, line 17 to read as follows:

--Figure 43A shows a schematic diagram of a structure in the amplicon derived from HCV type 1a (residues 136 to 213 of SEQ ID NO: 124) aligned with bridging probe having two seven-nucleotide regions of complementarity (SEQ ID NO:120). The regions that are complementary as aligned to the target are indicated by a black line between the strands.--.

Please amend the paragraph beginning at page 64, line 22 to read as follows:

--Figure 43B shows a schematic diagram of a structure in the amplicon derived from HCV type 1b (residues 22 to 125 of SEQ ID NO: 125) aligned with bridging probe having two 7 or 8 nucleotide regions of complementarity (SEQ ID NOS:121 and 122, respectively). The regions that are complementary as aligned to the target are indicated by a black line between the strands.--.

Please amend the paragraph beginning at page 84, line 12 to read as follows:

--Efficient screening of the suboptimal structures can be accomplished by incorporating constraints derived from experimental data or phylogenetic analysis into the computer algorithm. The use of structure specific nucleases having well characterized specificity have an advantage that the site of cleavage can convey additional information based on the structural requirements for cleavage. This is illustrated here by discussion of information

potentially gained by cleavage with a 5' nuclease, Cleavase® I nuclease, but the same deductive approach is equally applicable and useful for other structure-specific cleavage agents for which a substrate structure is well defined (*i.e.*, it is known where in the structure the cleavage can occur). The specificity of Cleavase® enzymes is such that cleavage occurs at the 5' ends of hairpin duplexes, after the first base pair (Lyamichev *et al., supra*). This means that any cleavage site identifies both a base that must be paired in the structure, and that the base to which it pairs must be downstream in the strand. This can expressed as follows: if there is a cleavage site at position *i*, then nucleotide *i* is base paired with nucleotide *j* where *j*>*i*. Entering into *mfold* the parameters *'f i* 0 2' and *'p i-i*+1 1-*i*-1' specifies that nucleotides *i* and *i*+1 should be basepaired to something (not to each other) and that *i* and *i*+1 can not be basepaired with nucleotides from 1 to *i*-1, respectively. This type of parameter can be considered a "soft" parameter because, while base pairing is required, the specific pairing partners of *i* and *i*+1 are left undefined, thereby allowing the suboptimal foldings generated using these parameters to predict multiple basepairing partners of these nucleotides. This allows the use of existing constraint parameters without modification of the folding algorithm to predict only those structures that correlate with the cleavage data. If cleavage occurs at position *i*, then a series of structures can be calculated to explain it using the following constraints, 'f *i* 0 1' (nucleotide *i* is forced to be base paired) and 'p 1 0 *i*-1' (prohibiting nucleotides from 1 to *i*-1 to be base paired). For example, to generate structures that could be responsible for a major cleavage site at position 90 of HCV1a DNA, folding of 244 nt DNA fragment of HCV1a (Figure 15) (SEQ ID NO: 124) was done using *mfold* version 2.3 (http://www.ibc.wustl.edu/~zuker) with constraints 'f 90 0 1' and 'p 1 0 89' predicting structure shown in Figure 16A (SEQ ID NO: 128). It is important that this structure not only predicts a cleavage site at position 90, but also explains cleavages at positions 102-103, 161 and 173, making it a good candidate to represent actual base pairing in the DNA molecule. The structure shown in Figure 16A (SEQ ID NO: 128) does not explain cleavage sites at positions 118-119 and 173. To reveal corresponding structures, the folding was done using constraints 'f 118 0 1' and 'p 1 0 117' (nucleotides 1-117 are not base paired and nucleotide 118 is base paired) with one of resulting structures shown in Figure 16B (SEQ ID NO: 128). Again this structure not only reasonably predicts cleavage site at position 117-

118 but also shows how cleavage at position 123 may happen. The same two structures were identified in the development of the experiments described in Example 8, using manual comparison of the cleavage sites and the 32 suboptimal folds. By either method, the knowledge of the structure specificity of the 5' nuclease made it possible to eliminate from consideration, all predicted structures that would require the cleavage sites to vary from the known substrate structure. This reduced the field of possible structures from 32 to 2. Use of additional enzymes, such as 3' nucleases, or duplex specific chemical agents, that can identify other positions that must be base-paired within a structure can further narrow the field.--.

Please amend the paragraph beginning at page 93, line 18 to read as follows:

--In alternative embodiments, contact sequences may be joined by synthesizing or otherwise creating a new oligonucleotide that incorporates both sequences into a single molecule. In one embodiment, the sequences are joined contiguously within the bridge oligonucleotide (*i.e.,* without any intervening nucleotides or other space-filling material). In another embodiment, the contact sequences are non-contiguous, with the spacing provided by additional nucleotides. In a preferred embodiment, the contact sequences are bridged by two thymidine nucleotides, as depicted in several of the bridging probes in Figure 11A (SEQ ID NOs: 50, 39, 42-44, and 47-49, respectively). In another preferred embodiment, the contact sequences in the bridging oligonucleotide are connected by a segment of nucleic acid containing a region of self-complementarity, such that the bridging oligonucleotide itself contains a folded structure. A stem-loop folded structure within the bridge oligonucleotide, if situated opposite a stem in the target nucleic acid, would permit the formation of a four-way Holliday structure, which is stabilized by coaxial stacking of the arms (Duckett *et al.,* Cell 55:79 [1988]).--.

Please amend the paragraph beginning at page 109, line 30 to read as follows:

--In this Example, the effects on oligonucleotide binding of either the formation of an occlusive structure, the presence of a single-base mismatch, or the presence of both at once were examined. To separate the effects on the efficiency of binding of structure from the effects of mismatches, four katG DNA target variants were chosen (SEQ ID NOS:1, 2, 3 and

4). The structures of these four targets in the region of the probe hybridization sites are shown in [Figure 2] Figures 2A-2D and the existence of the large stem-loop in structures 2C and 2D (SEQ ID NOS:3 and 4, respectively) was confirmed by digestion with the structure-specific Cleavase®I nuclease (Third Wave) and the cleavage sites are indicated by the arrows on structures 2C and 2D. The dark bar on the left of each structure in [Figure 2] Figures 2A-2D indicates the region to which the capture probe is expected to bind. The pointed kink in the black bar in structures 2B and 2D indicates a site of mismatch between the capture probe and the katG target.--.

Please amend the paragraph beginning at page 112, line 12 to read as follows:

--CFLP® reactions were performed on each 5'-TET labeled amplification product from the four KatG variants (2A-2D). Each CFLP® reaction contained approximately 20 fmole of the amplified product, 50 units of Cleavase® I nuclease in 10 µl of 1X CFLP® buffer (10 mM MOPS pH 7.5, 0.05% Tween® 20 and 0.05% Nonidet® P40 non-ionic detergents) with 0.2 mM $MnCl_2$. Reactions were assembled with all components except the enzyme and the $MnCl_2$, heated to 95°C for 15 seconds, then cooled to the reaction temperature of 50°C. The cleavage reactions were started with the addition of the enzyme and the $MnCl_2$, and incubated for 5 minutes. The reactions were terminated by the addition of 4 ml of 95% formamide with 10 mM EDTA and 0.02% Methyl Violet. The products were heated at 95°C for 30 sec, and aliquots were resolved by electrophoresis through 10% denaturing polyacrylamide gel (19:1 cross link) with 7 M urea in a buffer of 45 mM Tris-Borate, pH 8.3, 1.4 mM EDTA. The gel was visualized using the FMBIO-100 Image Analyzer (Hitachi). The resulting image is shown in the left panel of Figure 3. Lanes A-D contain CFLP reaction products from reactions containing structures 2A-2D, respectively. Lanes C and D contain a product (37 nt; indicated by the arrowhead) not present in lanes A and B which indicates the presence of the large stem-loop in structures 2C and 2D shown, respectively in [Figure 2] Figures 2C and 2D.--.

Please amend the paragraph beginning at page 113, line 2 to read as follows:

--In these experiments, the capture probes are bound to the target DNAs in solution and then immobilized on a solid support. The 391 bp fragment of katG described above was created by PCR using a 5'-fluorescein labelled primer (SEQ ID NO:7). A hybridization mixture was assembled, containing 40 fmoles of heat-denatured, 391 bp katG PCR product having one of the four sequences depicted in [Figure 2] Figures 2A-2D (SEQ ID NOS:1-4), labelled on the 5' end of the sense strand, 1.5 pmole of the biotinylated capture probe (SEQ ID NO:10), 0.01 mg/ml tRNA, 0.2% acetylated BSA, 4.5X SSPE and H_2O to 150 µl.--.

Please amend the paragraph beginning at page 127, line 1 to read as follows:

--This stepwise approach is illustrated here for a 244 nt amplicon derived from HCV type 1a. The identification of the cleavage sites in all four types of HCV amplicon is described in Example 3. Figure 15 shows sequence of 5' UTR region of HCV genotypes 1a (SEQ ID NO: 124), 1b (SEQ ID NO: 125), 2a/c (SEQ ID NO: 126) and 3a (SEQ ID NO: 127) with marked cleavage sites. Note that the designations 2a and 2a/c are used interchangeably throughout, and refer to the same HCV viral type, the amplicon of which is SEQ ID NO:22.--.

Please amend the paragraph beginning at page 127, line 7 to read as follows:

--The type 1a sequence as then subjected to folding predictions using the *mfold* version 2.3 program, which is available either through Genetics Computer Group (Madison, WI) or through public access to the authors' web site (http://www.ibc.wustl.edu/~zuker). Folding was done with using either DNA or RNA parameters with a selected folding temperature of 37°C. The output was set to include the optimal structure (lowest free energy) and any structure with a 20 percent or lower increase in calculated free energy (termed a "suboptimality of 20%"). All other program parameters used the default values. Folding with the RNA parameters generated 32 possible structures, while the DNA parameters gave 18 structures. Two of the structures predicted with the RNA parameters showed the best agreement with the cleavage data from the CFLP® analysis. These structures, the first and the thirtieth out of 32, are depicted in Figures 16A (SEQ ID NO: 128) and 16B (SEQ ID NO: 128).--.

Please amend the paragraph beginning at page 127, line 20 to read as follows:

--Structures predicted by the above analysis can be confirmed through the use of CFLP® analysis on fragments that delete the putative downstream pairing partner (Brow *et al., supra*). This approach, termed PCR walking, is illustrated here by the confirmation of the pairing partner responsible for the CFLP® cleavage at position 161 in the HCV type 1a 244 nt amplicon. The *mfold* program predicted a structure that paired a G at 161 with a C at position 205 (Figure 17A, left conformer). To confirm this two deletion amplicons were made. Each amplicon was 205 nt long. One included the C205 at the 3' end, while the other substituted a T at 205 to disrupt the basepair. PCR was conducted as described in Example 3, except the downstream primers 67 and 68 were substituted for (SEQ ID NO:25) used to amplify the full length amplicons. The resulting DNAs were purified and subjected to CFLP® analysis, resolved and visualized as described in Example 3. The resulting image is shown in Figure 17B (SEQ ID NO: 128). The identity of residue 205 in the deletion fragments is indicated above each lane, and the sizes of selected cleavage bands, as determined by comparison to a sequencing ladder in Example 3, are indicated on the right.--.

Please amend the paragraph beginning at page 150, line 23 to read as follows:

--Structure analysis of this amplicon using the *mfold* 2.3 software without any added constraints from the CFLP® pattern yielded only seven possible structures. Given the small number, manual analysis was sufficient to select from these 2 variants that together accounted for the major cleavage products seen in Figure 36. The cleavage sites are indicated on structures shown in Figure 37A (SEQ ID NO: 72) (structures generated used the hard constraints from PCR walking data, described below).--.

Please amend the paragraph beginning at page 150, line 29 to read as follows:

--The structure and cleavage analysis of the structure(s) contributing to the CFLP® band at position 62 are used here to demonstrate the next steps of the process. In both of the structures shown in Figure 37A (SEQ ID NO: 72), the C at nucleotide 62 is indicated to basepair with a G at nucleotide 114. The stem formed between these positions is the same in both structures, and is reproduced at the top of Figure 38A. One step in confirming the

interaction between these bases is to create a truncated version of this strand in which nucleotide 114 is changed to prevent pairing with nucleotide 62, and examine the resulting CFLP® cleavage (this is termed "PCR walking" in this discussion). This is shown schematically as the variant number 2 (SEQ ID NO: 90), the center structure at the bottom of Figure 37B. A control molecule that is similarly truncated, but that retains the putative 62/114 base pair is shown on the left as variant 1 (SEQ ID NO: 88). The CFLP® patterns from these 2 molecules are shown in the gel image at the right of Figure 37B, with an arrow indicating the band at position 62. It can be seen by the data in the first lane that the CFLP® pattern gives a strong signal at position 62 in the truncated control, confirming that nucleotide 62 does not require any of the material downstream of nt 114 (deleted in this construct) to basepair. Analysis of the variant with the disrupted basepair in lane 2 shows that removal of the 62/114 basepair shifts cleavage by one position, to the 63/113 basepair. Further variation to remove the 63/113 pairing, by changing nucleotide 113 as diagrammed in variant 3 (SEQ ID NO: 92) on the right, nearly eliminates this short stem region, and eliminates this particular CFLP® band from the pattern altogether (lane 3; the factors contributing to the slight residual signal at this position will be discussed below). This shows how the combination of truncation and mutation combined with CFLP® cleavage can be used to interrogate and confirm specific basepairs within predicted structures, thereby allowing their use as "hard constraints" in further computer-based modeling. The structures shown in Figure 37A (SEQ ID NO: 72) were generated using the hard constraints determined by such PCR walking. It is not required that further computer analysis be done before bridging probes are designed. If desired, bridge probes may be designed on the strength of the PCR walking data.--.

Please amend the paragraph beginning at page 152, line 19 to read as follows:

--The oligonucleotides designed to bind this stem are shown schematically in Figure 37C, aligned with the 62/114 structure (residues 54 to 122 of SEQ ID NO: 72). Several different approaches were used to link the contact sequences, including direct linkage without a spacer (shown as a gap in oligonucleotide 62-114b; SEQ ID NO:105), several different dinucleotides, as shown (62-114a [SEQ ID NO:106]; 62-114c [SEQ ID NO:107]; 62-114d

[SEQ ID NO:108]), or d-spacers (62-114e [SEQ ID NO:109]) (Glen Research Corp. (Sterling, VA)), indicated as "D"s, using one D for each spacer group (*i.e.*, DD indicates two such spacers used in sequence).--.

NOTE: In this instance please disregard the brackets as they are not intended to indicate the removal of the bracketed text.

Please amend the paragraph beginning at page 158, line 18 to read as follows:

--Using the structure analysis methods described above, new bridging oligonucleotides were designed for the target HCV 244bp DNA, which is the same target used before. One set of probes was designed to span a structure predicted to form with a base pair between 161 and 205 (Figure 43A) (residues 136 to 213 of SEQ ID NO: 124), while the other was designed to span a newly identified structure formed with the base pair between 33 and 77 (Figure 43B) (residues 22 to 125 of SEQ ID NO: 125).--.

In The Claims:

Please cancel Claims 1-89 without prejudice.

Please add the following Claims:

90. A method, comprising:

a) providing:

i) target nucleic acid comprising first and second non-contiguous single-stranded regions separated by an intervening region comprising a double-stranded region, wherein said target nucleic comprises at least a portion of Hepatitis C virus nucleic acid;

ii) a bridging oligonucleotide capable of binding to said first and second non-contiguous single-stranded regions;

iii) a second oligonucleotide capable of binding to a portion of said first non-contiguous single-stranded region; and

iii) a cleavage agent;

b) mixing said target nucleic acid, said bridging oligonucleotide, said second oligonucleotide, and said cleavage agent under conditions such that either said second oligonucleotide or said bridging oligonucleotide is cleaved.

91. The method of Claim 90, wherein said cleavage agent comprises a nuclease.

92. The method of Claim 91, wherein said cleavage agent comprises a thermostable 5' nuclease.

93. The method of Claim 92, wherein said thermostable 5' nuclease comprises an altered polymerase derived from a native polymerases of *Thermus* species.

94. The method of Claim 91, wherein said nuclease is selected from the group consisting of *Pyrococcus woesii* FEN-1 endonuclease, *Methanococcus jannaschii* FEN-1 endonuclease, *Pyrococcus furiosus* FEN-1 endonuclease, and *Archaeoglobus fulgidus* FEN-1 endonuclease.

95. The method of Claim 90, wherein said conditions of said mixing allow for hybridization of said bridging oligonucleotide and said second oligonucleotide to said target nucleic acid so as to define a region of overlap of said oligonucleotides.

96. The method of Claim 95, wherein said region of overlap comprises one base.

97. The method of Claim 95, wherein said region of overlap comprises more than one base.

98. The method of Claim 90, wherein said Hepatitis C virus is selected from the group consisting of Hepatitis C virus variants 1a, 1b, 2a/c, and 3a.

99. A method, comprising:

a) providing:

i) target nucleic acid comprising first and second non-contiguous single-stranded regions separated by an intervening region, said intervening region comprising a first double-stranded portion and a second double-stranded portion separated by a connecting single-stranded portion, wherein said target nucleic acid comprises at least a portion of Hepatitis C virus nucleic acid; and

ii) a bridging oligonucleotide capable of binding to said first and second non-contiguous single-stranded regions; and

b) mixing said target nucleic acid and said bridging oligonucleotide under conditions such that said bridging oligonucleotide hybridizes to said target to form an oligonucleotide/target complex.

100. The method of Claim 99, wherein said Hepatitis C virus is selected from the group consisting of Hepatitis C virus variants 1a, 1b, 2a/c, and 3a.

101. A method, comprising:

a) providing:

i) target nucleic acid comprising first and second non-contiguous single-stranded regions separated by an intervening region comprising a double-stranded portion, wherein said target nucleic acid comprises at least a portion of Hepatitis C virus nucleic acid;

ii) a bridging oligonucleotide capable of binding to said first and second non-contiguous single-stranded regions; and

iii) a reactant selected from the group consisting of polymerases and ligases; and

b) mixing said target nucleic acid, said bridging oligonucleotide and said reactant under conditions such that said bridging oligonucleotide is modified to produce a modified oligonucleotide.

102. The method of Claim 101, wherein said reactant is a polymerase, and said modified oligonucleotide comprises an extended oligonucleotide.

103. The method of Claim 101, wherein said reactant is a ligase, and said modified oligonucleotide comprises a ligated oligonucleotide.

104. The method of Claim 101, wherein said bridging oligonucleotide is capable of binding to fewer than ten nucleotides of each of said first and second non-contiguous single-stranded regions.

105. The method of Claim 104, wherein said bridging oligonucleotide is capable of binding to seven or fewer nucleotides of each of said first and second non-contiguous single-stranded regions.

106. The method of Claim 101, wherein said Hepatitis C virus is selected from the group consisting of Hepatitis C virus variants 1a, 1b, 2a/c, and 3a.

107. A method for detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products comprising:

a) providing:

i) a cleavage agent;

ii) Hepatitus C virus target nucleic acid, said target nucleic acid comprising a first region and a second region, said second region downstream of and contiguous to said first region;

iii) a first oligonucleotide, wherein at least a portion of said first oligonucleotide is completely complementary to said first portion of said first target nucleic acid;

iv) a second oligonucleotide comprising a 3' portion and a 5' portion, wherein said 5' portion is completely complementary to said second portion of said target nucleic acid;

b) mixing said cleavage agent, said target nucleic acid, said first oligonucleotide and said second oligonucleotide to create a reaction mixture under reaction conditions such that at least said portion of said first oligonucleotide is annealed to said first region of said target nucleic acid and wherein at least said 5' portion of said second oligonucleotide is annealed to said second region of said target nucleic acid so as to create a cleavage structure, and wherein cleavage of said cleavage structure occurs to generate non-target cleavage product; and

c) detecting the cleavage of said cleavage structure.

108. The method of Claim 107, wherein said detecting the cleavage of said cleavage structure comprises detecting said non-target cleavage product.

109. The method of Claim 107, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

110. The method of Claim 107, wherein said 3' portion of said second oligonucleotide consists of a single nucleotide not complementary to said target nucleic acid.

111. The method of Claim 107, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence.

112. The method of Claim 107, wherein said detecting the cleavage of said cleavage structure comprises detection of mass.

113. The method of Claim 107, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence energy transfer.

114. The method of Claim 107, wherein said detecting the cleavage of said cleavage structure comprises detection selected from the group consisting of detection of radioactivity, luminescence, phosphorescence, fluorescence polarization, and charge.

115. The method of Claim 107, wherein said first oligonucleotide is attached to a solid support.

116. The method of Claim 107, wherein said second oligonucleotide is attached to a solid support.

117. The method of Claim 107, wherein said cleavage agent comprises a structure-specific nuclease.

118. The method of Claim 117, wherein said structure-specific nuclease comprises a thermostable structure-specific nuclease.

119. The method of Claim 118, wherein said cleavage agent comprises a 5' nuclease.

120. The method of Claim 119, wherein said 5'-nuclease comprises a thermostable 5'-nuclease.

121. The method of Claim 120, wherein a portion of the amino acid sequence of said nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.

122. The method of Claim 121, wherein said thermophilic organism is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus*, and *Thermus thermophilus*.

123. The method of Claim 107, wherein said detecting the cleavage of said cleavage structure comprises:

a) providing:

i) said non-target cleavage product;

ii) a composition comprising two single-stranded nucleic acids annealed so as to define a single-stranded portion of a protein binding region; and

iii) a protein; and

b) exposing said non-target cleavage product to said single-stranded portion of said protein binding region under conditions such that said protein binds to said protein binding region.

124. The method of Claim 123, wherein said protein comprises a nucleic acid producing protein and wherein said nucleic acid producing protein binds to said protein binding region and produces nucleic acid.

125. The method of Claim 124, wherein said protein binding region is a template-dependent RNA polymerase binding region.

126. The method of Claim 125, wherein said template-dependent RNA polymerase binding region is a T7 RNA polymerase binding region.

127. The method of Claim 107, wherein said detecting the cleavage of said cleavage structure comprises:

a) providing:

i) said non-target cleavage product;

ii) a single continuous strand of nucleic acid comprising a sequence defining a single strand of an RNA polymerase binding region;

iii) a template-dependent DNA polymerase; and

iv) a template-dependent RNA polymerase;

b) exposing said non-target cleavage product to said RNA polymerase binding region under conditions such that said non-target cleavage product binds to a portion of said single strand of said RNA polymerase binding region to produce a bound non-target cleavage product;

c) exposing said bound non-target cleavage product to said template-dependent DNA polymerase under conditions such that a double-stranded RNA polymerase binding region is produced; and

d) exposing said double-stranded RNA polymerase binding region to said template-dependent RNA polymerase under conditions such that RNA transcripts are produced.

128. The method of Claim 127, further comprising the step of e) detecting said RNA transcripts.

129. The method of Claim 127, wherein said template-dependent RNA polymerase is T7 RNA polymerase.

130. The method of Claim 107, wherein said target nucleic acid comprises single-stranded DNA.

131. The method of Claim 107, wherein said target nucleic acid comprises double-stranded DNA and prior to step c), said reaction mixture is treated such that said double-stranded DNA is rendered substantially single-stranded.

132. The method of Claim 131, wherein said double-stranded DNA is rendered substantially single-stranded by heat.

133. The method of Claim 107, wherein said reaction conditions comprise providing a source of divalent cations.

134. The method of Claim 133, wherein said divalent cation is selected from the group consisting of Mn^{2+} and Mg^{2+} ions.

135. The method of Claim 107, wherein said first and said second oligonucleotides are provided in concentration excess compared to said target nucleic acid.

136. The method of Claim 107, further comprising providing a third oligonucleotide complementary to a third portion of said target nucleic acid upstream of said first portion of said first target nucleic acid, wherein said third oligonucleotide is mixed with said reaction mixture in step b).

Appendix 2

90. A method, comprising:

a) providing:

i) target nucleic acid comprising first and second non-contiguous single-stranded regions separated by an intervening region comprising a double-stranded region, wherein said target nucleic comprises at least a portion of Hepatitis C virus nucleic acid;

ii) a bridging oligonucleotide capable of binding to said first and second non-contiguous single-stranded regions;

iii) a second oligonucleotide capable of binding to a portion of said first non-contiguous single-stranded region; and

iii) a cleavage agent;

b) mixing said target nucleic acid, said bridging oligonucleotide, said second oligonucleotide, and said cleavage agent under conditions such that either said second oligonucleotide or said bridging oligonucleotide is cleaved.

91. The method of Claim 90, wherein said cleavage agent comprises a nuclease.

92. The method of Claim 91, wherein said cleavage agent comprises a thermostable 5' nuclease.

93. The method of Claim 92, wherein said thermostable 5' nuclease comprises an altered polymerase derived from a native polymerases of *Thermus* species.

94. The method of Claim 91, wherein said nuclease is selected from the group consisting of *Pyrococcus woesii* FEN-1 endonuclease, *Methanococcus jannaschii* FEN-1 endonuclease, *Pyrococcus furiosus* FEN-1 endonuclease, and *Archaeoglobus fulgidus* FEN-1 endonuclease.

95. The method of Claim 90, wherein said conditions of said mixing allow for hybridization of said bridging oligonucleotide and said second oligonucleotide to said target nucleic acid so as to define a region of overlap of said oligonucleotides.

96. The method of Claim 95, wherein said region of overlap comprises one base.

97. The method of Claim 95, wherein said region of overlap comprises more than one base.

98. The method of Claim 90, wherein said Hepatitis C virus is selected from the group consisting of Hepatitis C virus variants 1a, 1b, 2a/c, and 3a.

99. A method, comprising:

a) providing:

i) target nucleic acid comprising first and second non-contiguous single-stranded regions separated by an intervening region, said intervening region comprising a first double-stranded portion and a second double-stranded portion separated by a connecting single-stranded portion, wherein said target nucleic acid comprises at least a portion of Hepatitis C virus nucleic acid; and

ii) a bridging oligonucleotide capable of binding to said first and second non-contiguous single-stranded regions; and

b) mixing said target nucleic acid and said bridging oligonucleotide under conditions such that said bridging oligonucleotide hybridizes to said target to form an oligonucleotide/target complex.

100. The method of Claim 99, wherein said Hepatitis C virus is selected from the group consisting of Hepatitis C virus variants 1a, 1b, 2a/c, and 3a.

101. A method, comprising:

a) providing:

i) target nucleic acid comprising first and second non-contiguous single-stranded regions separated by an intervening region comprising a double-stranded portion, wherein said target nucleic acid comprises at least a portion of Hepatitis C virus nucleic acid;

ii) a bridging oligonucleotide capable of binding to said first and second non-contiguous single-stranded regions; and

iii) a reactant selected from the group consisting of polymerases and ligases; and

b) mixing said target nucleic acid, said bridging oligonucleotide and said reactant under conditions such that said bridging oligonucleotide is modified to produce a modified oligonucleotide.

102. The method of Claim 101, wherein said reactant is a polymerase, and said modified oligonucleotide comprises an extended oligonucleotide.

103. The method of Claim 101, wherein said reactant is a ligase, and said modified oligonucleotide comprises a ligated oligonucleotide.

104. The method of Claim 101, wherein said bridging oligonucleotide is capable of binding to fewer than ten nucleotides of each of said first and second non-contiguous single-stranded regions.

105. The method of Claim 104, wherein said bridging oligonucleotide is capable of binding to seven or fewer nucleotides of each of said first and second non-contiguous single-stranded regions.

106. The method of Claim 101, wherein said Hepatitis C virus is selected from the group consisting of Hepatitis C virus variants 1a, 1b, 2a/c, and 3a.

107. A method for detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products comprising:

a) providing:

i) a cleavage agent;

ii) Hepatitus C virus target nucleic acid, said target nucleic acid comprising a first region and a second region, said second region downstream of and contiguous to said first region;

iii) a first oligonucleotide, wherein at least a portion of said first oligonucleotide is completely complementary to said first portion of said first target nucleic acid;

iv) a second oligonucleotide comprising a 3' portion and a 5' portion, wherein said 5' portion is completely complementary to said second portion of said target nucleic acid;

b) mixing said cleavage agent, said target nucleic acid, said first oligonucleotide and said second oligonucleotide to create a reaction mixture under reaction conditions such that at least said portion of said first oligonucleotide is annealed to said first region of said target nucleic acid and wherein at least said 5' portion of said second oligonucleotide is annealed to said second region of said target nucleic acid so as to create a cleavage structure, and wherein cleavage of said cleavage structure occurs to generate non-target cleavage product; and

c) detecting the cleavage of said cleavage structure.

108. The method of Claim 107, wherein said detecting the cleavage of said cleavage structure comprises detecting said non-target cleavage product.

109. The method of Claim 107, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

110. The method of Claim 107, wherein said 3' portion of said second oligonucleotide consists of a single nucleotide not complementary to said target nucleic acid.

111. The method of Claim 107, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence.

112. The method of Claim 107, wherein said detecting the cleavage of said cleavage structure comprises detection of mass.

113. The method of Claim 107, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence energy transfer.

114. The method of Claim 107, wherein said detecting the cleavage of said cleavage structure comprises detection selected from the group consisting of detection of radioactivity, luminescence, phosphorescence, fluorescence polarization, and charge.

115. The method of Claim 107, wherein said first oligonucleotide is attached to a solid support.

116. The method of Claim 107, wherein said second oligonucleotide is attached to a solid support.

117. The method of Claim 107, wherein said cleavage agent comprises a structure-specific nuclease.

118. The method of Claim 117, wherein said structure-specific nuclease comprises a thermostable structure-specific nuclease.

119. The method of Claim 118, wherein said cleavage agent comprises a 5' nuclease.

120. The method of Claim 119, wherein said 5'-nuclease comprises a thermostable 5'-nuclease.

121. The method of Claim 120, wherein a portion of the amino acid sequence of said nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.

122. The method of Claim 121, wherein said thermophilic organism is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus*, and *Thermus thermophilus*.

123. The method of Claim 107, wherein said detecting the cleavage of said cleavage structure comprises:

a) providing:

i) said non-target cleavage product;

ii) a composition comprising two single-stranded nucleic acids annealed so as to define a single-stranded portion of a protein binding region; and

iii) a protein; and

b) exposing said non-target cleavage product to said single-stranded portion of said protein binding region under conditions such that said protein binds to said protein binding region.

124. The method of Claim 123, wherein said protein comprises a nucleic acid producing protein and wherein said nucleic acid producing protein binds to said protein binding region and produces nucleic acid.

125. The method of Claim 124, wherein said protein binding region is a template-dependent RNA polymerase binding region.

126. The method of Claim 125, wherein said template-dependent RNA polymerase binding region is a T7 RNA polymerase binding region.

127. The method of Claim 107, wherein said detecting the cleavage of said cleavage structure comprises:

a) providing:

i) said non-target cleavage product;

ii) a single continuous strand of nucleic acid comprising a sequence defining a single strand of an RNA polymerase binding region;

iii) a template-dependent DNA polymerase; and

iv) a template-dependent RNA polymerase;

b) exposing said non-target cleavage product to said RNA polymerase binding region under conditions such that said non-target cleavage product binds to a portion of said single strand of said RNA polymerase binding region to produce a bound non-target cleavage product;

c) exposing said bound non-target cleavage product to said template-dependent DNA polymerase under conditions such that a double-stranded RNA polymerase binding region is produced; and

d) exposing said double-stranded RNA polymerase binding region to said template-dependent RNA polymerase under conditions such that RNA transcripts are produced.

128. The method of Claim 127, further comprising the step of e) detecting said RNA transcripts.

129. The method of Claim 127, wherein said template-dependent RNA polymerase is T7 RNA polymerase.

130. The method of Claim 107, wherein said target nucleic acid comprises single-stranded DNA.

131. The method of Claim 107, wherein said target nucleic acid comprises double-stranded DNA and prior to step c), said reaction mixture is treated such that said double-stranded DNA is rendered substantially single-stranded.

132. The method of Claim 131, wherein said double-stranded DNA is rendered substantially single-stranded by heat.

133. The method of Claim 107, wherein said reaction conditions comprise providing a source of divalent cations.

134. The method of Claim 133, wherein said divalent cation is selected from the group consisting of Mn^{2+} and Mg^{2+} ions.

135. The method of Claim 107, wherein said first and said second oligonucleotides are provided in concentration excess compared to said target nucleic acid.

136. The method of Claim 107, further comprising providing a third oligonucleotide complementary to a third portion of said target nucleic acid upstream of said first portion of said first target nucleic acid, wherein said third oligonucleotide is mixed with said reaction mixture in step b).